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☐	L1	helicobacter.ti,ab,clm. and (atccr3504 or 43504)	95
☐	L2	helicobacter.ti,ab,clm. and (atcc43504 or 43504)	113
☐	L3	helicobacter.ti,ab,clm. and (atcc-43504 or 43504)	95
☐	L4	(l1 or l2 or l3) and ((crossreact$ or cross-react$ or non-specific or shared or common or nonspecific$) near10 (protein or antigen or assay or immunoassay))	17

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J Chromatogr B Biomed Sci Appl. 1998 Sep 4;714(2):325-33. Related Articles, Links

Characterization of a Helicobacter pylori vaccine candidate by proteome techniques.

McAtee CP, Lim MY, Fung K, Velligan M, Fry K, Chow TP, Berg DE.

Genelabs Technologies, Redwood City, CA 94063, USA.

In a previous two-dimensional (2D) gel electrophoretic study of protein antigens of the gastric pathogen, Helicobacter pylori recognized by human sera, one of the highly and consistently reactive antigens, a protein with Mr of approximately 30,000 (Spot 15) seemed to be of special interest because of low yields on N-terminal protein sequencing. This suggested possible N-terminal modification, as the N-terminal sequence analysis of this 30,000 protein (Spot 15) did not provide a definitive match within the H. pylori genomic database. This protein was isolated by 2D polyacrylamide gel electrophoresis, evaluated by liquid chromatography-mass spectrometry, and found to consist of two related species of approximately 28,100 and 26,500. In parallel, the proteins within this spot were digested in situ with the endoprotease Lys-C. Analysis of the Lys-C digest by matrix-assisted laser desorption time-of-flight mass spectrometry, peptide mapping, and sequence analysis was conducted. Comparison of the mass and sequence of the Lys-C peptides with those derived from a H. pylori genomic library identified an open reading frame of approximately 300 base pairs as the source of the Spot 15 protein. This corresponded to HP0175 in the recently reported H. pylori genome sequence, an open reading frame with some homology to Campylobacter jejeuni cell binding protein 2. Mass spectral and sequence analysis indicated that Spot 15 was a processed product generated by proteolytic cleavage at both the carboxy and amino termini of the 34 open reading frame precursor.

Afr Med J. 1991 Dec 7;80(11-12):575-8. Related Articles, Links

Evaluation of various laboratory techniques to diagnose Helicobacter pylori in patients with upper gastro-intestinal tract symptoms.

Miller NM, Sathar MA, Naran AD, van den Ende J, Simjee AE, Manion G.

Department of Medical Microbiology, University of Natal, Durban.

Helicobacter (Campylobacter) pylori is strongly associated with type B gastritis. The detection of H. pylori, which entails histological examination and culture of gastric biopsy specimens, takes several days. There has been much interest in developing more rapid tests, including non-invasive ones. Using histology and/or culture as the 'gold standard', several methods to detect H. pylori were compared and evaluated. The organism was detected in 84 of 100 consecutive patients attending the Gastrointestinal Unit of King Edward VIII Hospital for upper gastrointestinal tract endoscopy. Histological examination was the most sensitive (98%) and specific (100%) method used in detecting H. pylori in gastric biopsy specimens. An enzyme-linked immunosorbent assay to detect specific IgG antibodies to whole H. pylori organisms is a moderately sensitive (82%), non-invasive method but it is nonspecific (38%). Although culture was specific (100%), it was less sensitive (68%) than histological examination. The 'conventional' urease assays must be performed under controlled conditions (37 degrees C) for optimal results (sensitivity, 71%).

PMID: 1745946 [PubMed - indexed for MEDLINE]

L4: Entry 7 of 17 File: PGPB Aug 7, 2003

DOCUMENT-IDENTIFIER: US 20030148411 A1
TITLE: Method for detecting helicobacter pylori and heilmanii in fecal and salivary specimen and biopsy material

Abstract Paragraph:
The invention relates to a method for detecting pathogenic organisms, in particular Helicobacter pylori and H.heilmanii, in fecal, salivary and secretory samples by means of a double-antibody sandwich assay. The inventive method is characterized by dissolving or dispersing the sample having the pathogen antigen in a buffer solution and contacting the buffer solution with a solid phase to which at least two primary antibodies are bound, one of which specifically binds to the pathogenic antigen and the other to human immunoglobulin A; washing the solid phase of non-specifically bound proteins and contacting the solid phase with a secondary antibody which specifically binds to pathogenic antigen, and determining the quantity of specifically bound secondary antibodies.

Detail Description Paragraph:
[0024] Detection of binding: Into the wells there was dosed in each case 100 .mu.l biotin-conjugated polyclonal rabbit-anti-H.pylori antibody (1:10000; DAKO, Hamburg) or horseradish peroxidase (HRP)-conjugated polyclonal goat-anti-H.pylori antibody (KPL, Kirkegaard and Perry Laboratories, Gaithersburg, MD; a mixture of polyclonal antibodies against the H.pylori types ATCC 43504, 43526, 43579) diluted 1:1000 in washing buffer, and incubated at room temperature for 1 hour while been vibrated. The solution was removed from the wells and each well washed five times with 200 .mu.l washing buffer.

Detail Description Paragraph:
[0031] The result of the mass screening is shown in FIG. 3 in a bar chart. The bar chart shows that patients with an H.pylori infection present in the saliva as a rule have more than 60 ng/ml H.pylori antigens. The testing of the saliva is thus suitable for the detection of the presence of an H.pylori infection. H.pylori antigen concentrations below 25 ng per ml saliva indicate, in contrast, a non-specific cross-reaction with other pathogens.

CLAIMS:

5. Method according to any of claims 1 to 4, wherein the first primary antibody and the secondary antibody bind Helicobacter pylori antigens.

6. Method according to any of claims 1 to 5, wherein the first primary antibody is a mixture of polyclonal and/or monoclonal antibodies against various Helicobacter pylori types.

7. Method according to any of claims 1 to 6, wherein the secondary antibody is a mixture of polyclonal and/or monoclonal antibodies against various Helicobacter pylori types.

9. Method according to any of claims 1 to 4, characterized in that the pathogen is Helicobacter heilmanii.

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☐ 1. Document ID: US 20050130239 A1

L4: Entry 1 of 17 File: PGPB Jun 16, 2005

DOCUMENT-IDENTIFIER: US 20050130239 A1
TITLE: Immunoassay for H. pylori in fecal specimens using genus specific monoclonal antibody

Abstract Paragraph:
The present invention provides a method for detecting H. pylori in fecal specimens. The present invention employs an immunoassay using genus specific monoclonal antibody to Helicobacter or Campylobacter on one side of the assay and H. pylori specific antibodies on the other side of the assay. The immunoassay will typically be supplied in the form of a kit including a plate of antibody-coated wells, sample diluent, the labeled antibody, e.g., an enzyme-antibody conjugate, wash buffer and, in the case of the ELISA, a substrate solution. Alternatively, one or more of the following assays can be used to detect the presence of the H. pylori antigen: an enzyme-linked assay, a radioimmunoassay, a fluorescence immunoassay, a chemiluminescent assay, a lateral flow assay, an agglutination assay, a particulate-based assay, an immunoprecipitation assay and an immunoblotting assay.

Summary of Invention Paragraph:
[0004] Several major antigens have been identified and used in immunoassays in the detection of H. pylori antibodies. However, these assays have not exhibited the specificity and sensitivity that are desired in serodiagnosis. Newwell, D. G., et al. Serodian. Immunother. Infec. Dis., 3:1-6 (1989). One problem with these immunoassays is cross-reactivity. Studies of the dominant antigens in H. pylori, in particular, the putative flagellar protein, which has a molecular weight of 60 Da, have shown that some of these antigen are not specific to H. pylori and also found in other bacteria such as C. jenui and C. coli. A second problem that has been encountered in designing immunoassays for H. pylori is strain variation. Substantial differences in the antigens have been observed in different strains of H. pylori. These problems preclude designing an assay around the use of a single antigen. One approach that has been taken to improving the specificity and selectivity of antibody immunoassays for H. pylori has been to use a mixture of antigens from different H. pylori strains which mixture is enriched with certain antigen fragments. One ELSIA which detects H. pylori antibodies in blood sera is commercially available from Meridian Diagnostics. This assay uses a bacterial whole cell lysate as the antigen.

Detail Description Paragraph:
[0020] H. pylori cells from ATCC strain 43504 have been found to be particularly useful in producing the polyclonal antibody. As previously mentioned, substantial strain variation has been observed in H. pylori. Differences in the organism have been observed in different geographic regions as well as dietary groups. However, antibodies obtained through sensitization using cells from strain 43504 have been found to be useful in detecting the organism across geographic regions and dietary

needed.

CLAIMS:

14. A process for the determination of H. pylori antigen in a human fecal specimen which comprises: (a) dispersing human fecal specimen in a sample diluent; (b) contacting the fecal specimen in the diluent with a first antibody to form a complex of the antibody and the antigen; (c) separating said specimen and said complex; (d) exposing the complex to a second antibody and a portion of the second antibody reacting with said complex, one of said first and second antibody being selected from the group consisting of polyclonal H. pylori antigen specific antibodies, a plurality of monoclonal H. pylori antigen specific antibodies and mixtures thereof; and the other of the first and second antibody being a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and also binds to H. pylori antigen, one of said first and second antibody being bound to a solid carrier and the other being labeled with a detection agent; and (e) detecting the amount of the labeled antibody in said complex and in turn determining the presence of H. pylori antigen in said fecal specimen.

23. The process of claim 1 wherein after exposing the complex to the second antibody, the complex is washed with a buffer that reduces cross-reactivity or otherwise improves the specificity of the assay.

24. A process for the determination of H. pylori in a fecal specimen which comprises: (a) dispersing a human fecal specimen in a diluent; (b) contacting the fecal specimen in the diluent with a first antibody reactive with H. pylori antigen bound to a solid carrier and a second labeled antibody reactive with H. pylori to form a complex of the antibodies and the antigen, one of said first and second antibody being selected from the group consisting of polyclonal H. pylori antigen specific antibodies, a plurality of H. pylori antigen specific monoclonal antibodies, and mixtures thereof and the other of the first and second antibody being a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and also binds to H. pylori antigen; (c) separating said specimen and said complex; (d) detecting the labeled antibody in said complex formed in step (b) and in turn determining the presence of H. pylori antigen in said fecal specimen.

25. A process for the determination of H. pylori in a fecal specimen which comprises: (a) dispersing a human fecal specimen in a sample diluent; (b) contacting the fecal specimen in the diluent with a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and binds to H. pylori antigen bound to a solid carrier to form a complex of the antibody and the antigen; (c) separating said specimen and said complex; (d) contacting the antibody-antigen complex formed in step (b) with a primary antibody specific for H. pylori antigen obtained from an antibody-producing species to produce an antibody-antigen-antibody complex; (e) removing the primary antibody not present in the complex from step (c); (f) contacting the antibody-antigen-antibody complex formed in step (e) with a secondary antibody, said secondary antibody being an antibody that specifically binds the antibody-producing species antibody, whereby said secondary antibody forms a complex with said antibody-antigen-antibody complex; and (g) determining the presence of H. pylori antigen in said fecal specimen by detecting the complex formed in step (f).

26. A kit for the determination of H. pylori in a fecal specimen including a plate of wells having bound thereto a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and also binds to H. pylori antigen, a protein-based sample diluent and a plurality of labeled antibodies selected from the group consisting of polyclonal H. pylori antigen specific antibodies, a plurality of monoclonal H. pylori antigen specific

groups. If necessary, for example, if it is found that the ELISA is not effective in detecting the organism in certain populations, cells from more than one strain of H. pylori could be used to produce the antibody.

Detail Description Paragraph:
[0023] To prepare the fecal specimen for use in the assay, the specimen is dispersed in a protein-based sample diluent. The diluent being formulated and buffered to minimize cross-reactivity. As examples of sample diluents, mention can be made of fetal bovine serum, normal goat serum, guinea pig serum, horse serum, casein, albumin, gelatin, and bovine serum albumin (BSA). A dilution of one part fecal specimen and four parts diluent has been found to be useful. In addition to using the protein based additives, cross-reactivity can be reduced by the addition of detergents and increasing or decreasing pH or ionic strength of the diluent buffer. For example, many sample diluents contain Triton X-100 and/or Tween 20 at concentrations ranging between 0.05% and 2%. NaCl can be added in the ranges between 0-2.9% to alter the ionic strength of the buffer system. These changes lead to greater specificity by reducing the likelihood of weak or non-specific interactions from forming.

Detail Description Paragraph:
[0024] Cross-reactivity can also be addressed in the formulation of the H. pylori specific antibody solutions and the washes that are used in the assay. The H. pylori specific antibody can be provided in a buffered solution in conjunction with one of the protein sera mentioned previously. The washes used in the assay can be formulated and buffered by the addition of salts and surfactants to control cross-reactivity. A preferred wash for reducing cross-reactivity is a phosphate buffered saline solution.

Detail Description Paragraph:
[0026] H. pylori (ATCC strain 43504) was streaked for isolation on Tryptic Soy Agar (TSA) supplemented with 5% defibrinated sheep blood. The plate was incubated at 37.degree. C. in a microaerophilic environment for 6-7 days. The resultant bacterial growth was evaluated by use of colony morphology, urease, catalase and oxidase reactions, and gram strain. Acceptable growth was subcultured to four TSA with sheep blood agar plates and grown at 37.degree. C. in a microaerophilic environment for 3-4 days.

Detail Description Paragraph:
[0037] So-called triple sandwich assays can also be used for detecting H. pylori in fecal specimens in accordance with the invention. Triple assays are known in the art and the basic methodology can be applied to the detection of H. pylori in fecal specimens. A triple assay is typically conducted by dispersing a fecal specimen suspected of carrying H. pylori in a sample diluent which minimizes cross-reactivity and adding the diluted sample to an immobilized genus specific monoclonal antibody. The sample is incubated to form the antibody-antigen complex. After washing excess specimen from the immobilized support, and H. pylori specific antibody known as a primary antibody and obtained from a species of an antibody producing animal is added to the antibody-antigen complex and incubated for form an antibody-antigen-antibody complex. After forming this complex and removing the unreacted antibody, the complex is reacted with an antibody known as a secondary antibody which is an antibody to the antibody producing species such as anti-(rabbit, cow or goat) immunoglobulin. The secondary antibody is labeled in a conventional manner, typically with an enzyme, an incubated with the antibody-antigen-antibody complex to form a triple antibody complex or sandwich. After removing the unreacted secondary antibody, the antigen is assayed in a conventional manner. In using an enzyme label, a substrate is added to the complex of the antigen and the three antibodies and the reaction of the substrate with the linked enzyme is monitored to determine the amount of the antigen present in the specimen. In the triple sandwich assay, as in the basic sandwich assay, the washes and the antibody solutions are formulated or buffered to control cross-reactivity as

antibodies and mixtures thereof.

28. A process for the determination of H. pylori in a fecal specimen which comprises: (a) dispersing a human fecal specimen in a sample diluent; (h) contacting the fecal specimen in the diluent with a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and binds to H. pylori antigen bound to a solid carrier to form a complex of the antibody and the antigen; (i) separating said specimen and said complex; (j) contacting the antibody-antigen complex formed in step (b) with a primary antibody for H. pylori antigen obtained from an antibody-producing species to produce an antibody-antigen-antibody complex; (k) removing the primary antibody not present in the complex from step (c); (l) contacting the antibody-antigen-antibody complex formed in step (e) with a secondary antibody, said secondary antibody being an antibody that specifically binds the antibody-producing species antibody, whereby said secondary antibody forms a complex with said antibody-antigen-antibody complex; and (m) determining the presence of H. pylori antigen in said fecal specimen by detecting the complex formed in step (f).

2. Document ID: US 20050048077 A1

L4: Entry 2 of 17 File: PGPB Mar 3, 2005

DOCUMENT-IDENTIFIER: US 20050048077 A1
TITLE: Compositions, test kits and methods for detecting helicobacter pylori

Summary of Invention Paragraph:
[0007] Serological tests and the .sup.13C UBT are the two non-invasive techniques, used in the management of H. pylori infection and eradication. The accuracy of a serological test is dependent on the nature of the antigen(s). Most of the serological tests are ELISA based and use whole cell lysates of H. pylori as the antigen, often in combination with a more purified antigen preparation like recombinant vacA, cagA and/or iceA protein. Using a crude lysate preparation of the whole organism can cause problems with the specificity of the test via nonspecific binding of antibodies not specific for H. pylori to components of the antigen preparation that might be present in other H. pylori related organisms (false positives). On the other hand crude antigen preparation might cause false negative results because unwanted components in the preparation might dilute specific antigens or interfere with the presentation of those re-quired to determine infection. The use of a total protein isolate also prevents serology from detecting loss of the organism and therefore is not suitable for evaluating success of eradication therapy. The UBT gives false negatives when patients are taking proton pump inhibitor drugs (PPI's) due to inhibition of urease activity by neutral pH.

Brief Description of Drawings Paragraph:
[0014] FIG. 1 is titled "Reactivities of H. pylori positive sera with antigens from Hp504" This figures show the average titers of specific antibodies, expressed as percent Integrated Optical density (IOD), with HP1, HP2, HP3 from H pylori strain Hp504 (ATCC#43504) present in sera from 9 patients diagnosed with a H. pylori infection achieved in two independent experiments. The serum samples from each patient were obtained before, 3 months and 5 months after eradication therapy.

Detail Description Paragraph:
[0058] H. pylori strain ATCC#43504 (Hp504)(American Type Culture Collection, Rockville, Md.) and two clinical isolates, Hp08 and Hp02, were used as the source of H. pylori proteins. As a control for the specificity of the serological reactivities Campylobacter jejuni strain ATCC#29428 was included into the experiments.

Detail Description Paragraph:
[0093] The following figures show the serial changes in titers of serum IgG expressed in % of integrated optical density that is left 3 months and 5 months after eradication treatment in comparison to the amount before treatment. The nine sera from with H. pylori infected and treated patients were tested on whole cell lysate separations of H. pylori strain ATCC#43504 (Hp504), Hp08 and Hp02 (clinical isolates). The results are shown for each tested bacterial strain separately to demonstrate that the accuracy of the test is independent of the source of the antigen.

CLAIMS:

1. A composition comprising three isolated and purified proteins, wherein the proteins are selected from the group consisting of HP1, HP2 and HP3, each of said proteins comprising regions which act as antigens specific to Helicobacter pylori, HP1 having of molecular weight of 32 kd, HP2 having of molecular weight of 30 kd and HP3 having of molecular weight of 23 kd, each of said proteins being derived from Helicobacter pylori bacteria and wherein HP1 has the sequence of 1 mkannhfkdf awkkcllgas vvallvgcsp hiietneval 61 klnyhpasek vqaldekill lrpafqysdn iakeyenkfk 121 nqtalkveqi lqnqgykvis vdssdkddls fsqkkegyla 181 vamngeivlr pdpkrtiqkk sepgllfstg ldkmegvlip 241 agfvkvtile pmsgesldsf tmdlseldiq ekflktthss 301 hsgglvstmv kgtdnsndai ksalnkifan imqeidkklt 361 qknlesyqkd akelkgkrnr (SEQUENCE ID NO. 1) HP2 has the sequence of 1 mkrssvfsfl vafllvagcs hkmdnktvag dvsaktvqta pvttepapek eepkqepapv 61 veekpavesg tiiasiyfdf dkyeikesdq etldeivqka kenhmqvlle gntdefgsse 121 ynqalgvkrt lsvknalvik gvekdmikti sfgetkpkca qktrecyken rrvdvklmk (SEQUENCE ID NO. 2), and HP3 has the sequence of 1 mleksflksk qlflcglgvl mlqactcpnt sqrnsflqdv pywmlqnrse yitqgvdssh 61 ivdgkkteei ekiatkrati rvaqnivhkl keaylsktnr ikqkitnemf iqmtqpiyds 121 lmnvdrlgiy inpnneevfa lvrargfdkd alseglhkms ldnqavsilv akveeifkds 181 vnygdvkvpi am (SEQUENCE ID NO. 3).

9. A method according to claim 8 wherein the Helicobacter pylori is Helicobacter pylori strain ATCC#43504.

10. A method for detecting the presence or absence of antibodies resulting from Helicobacter pylori infection in a biological sample, the method comprising contacting the sample with a composition according to claim 1; permitting the sample and said composition to form an antigen-antibody complex with respect to any antibody contained in the sample which is specific to the antigens included in the proteins of the composition; detecting the presence or absence of any formed antigen-antibody complex thereby learning of the presence or absence of Helicobacter pylori infection.

15. A kit for determining the presence of antibodies formed in response to Helicobacter pylori infection in a biological sample, the kit comprising a composition according to claim 1.

20. In a method for determining the eradication of Helicobacter pylori the improvement consisting of the detection of the presence or absence of antibodies resulting from Helicobacter pylori infection by a method according to claim 10, before, during and after eradication treatment.

3. Document ID: US 20040138415 A1

L4: Entry 3 of 17 File: PGPB Jul 15, 2004

DOCUMENT-IDENTIFIER: US 20040138415 A1
TITLE: Helicobacter proteins, nucleic acids and uses thereof

Abstract Paragraph:
The invention discloses Helicobacter HP30 or HP56 polypeptide, polypeptides derived thereof (HP30-derived or HP56-derived polypeptides), nucleic acids encoding said polypeptides, antibodies that specifically bind the HP30, HP56, HP30-derived or HP56-derived polypeptides and T cells specific for HP30, HP56, HP30-derived or HP56-derived polypeptide. Also disclosed are prophylactic or therapeutic compositions, including immunogenic compositions, e.g. vaccines, comprising HP30, HP56, HP30-derived or HP56-derived polypeptides, nucleic acids encoding the same or antibodies thereto. The invention additionally discloses methods of inducing in animals an immune response to Helicobacter cells or antigens.

Summary of Invention Paragraph:
[0019] Preferably, the HP30- or HP56-derived polypeptides of the invention are immunologically cross-reactive with the H. pylori peptide/protein from which they are derived, and are capable of eliciting in an animal an immune response to H. pylori. A preferred HP30- or HP56-derived polypeptide of the invention induces IgM, IgG, IgA, IgE antibodies, a delayed hypersensitivity T cell response and/or cytotoxic T cell response to cells expressing H. pylori antigen (including but not limited to antigen presenting cells such as macrophages, dendritic cells, B cells, or synthetic antigen presenting cells which display H. pylori antigen), native HP30 or HP56 protein from which the polypeptide is derived, H. pylori cells, or H. pylori cell lysate.

Detail Description Paragraph:
[0087] Strains from any of these organism may be obtained worldwide from any biologicals depository, particularly ATCC deposited strains of Helicobacter 43504, 43504D, 43526, 49503, 51652, 51653, 51932, 700392, 700392D 700824D, 51110, 51111, 51407, 51652, 51653, 700392, 700392D, 43504, 43504D, 43526, 43579, 49503, 51110, 51111, 51407, 51211, 51480, 51482, 51630, 51631, 51632, 51800, 51801, 51802, 51863, 51864, 700030, 700031, 700242, 700932, 49286, 49396, 49615, 51101, 51102, 51103, 51104, 51212, 51401, 51402, 51448, 51449, 51450, 51478, 51480, 51482, 51630, 51632, 51800, 51801, 51802, 51863, 51864, 51932, 700030, 700031, 700242, 700824D and 700932.

Detail Description Paragraph:
[0134] The HP56 or HP30 polypeptide of the invention may be isolated from a protein extract including a whole cell extract, of any Helicobacter spp., including, but hot limited to, Helicobacter pylori or Helicobacter felis. Strains from any of these organisms may be obtained worldwide from any biologicals depository, particularly strains of ATCC 43504D, 43526, 49503, 51652, 51653, 51932, 700392, 700392D 700824D, 51110, 51111, 51407, 51652, 51653, 700392, 700392D, 43504, 43504D, 43526, 43579, 49503, 51110, 51111, 51407, 51211, 51480, 51482, 51630, 51631, 51632, 51800, 51801, 51802, 51863, 51864, 700030, 700031, 700242, 700932, 49286, 49396, 49615, 51101, 51102, 51103, 51104, 51212, 51401, 51402, 51448, 51449, 51450, 51478, 51480, 51482, 51630, 51632, 51800, 51801, 51802, 51863, 51864, 51932, 700030, 700031, 700242, 700824D and 700932.

Detail Description Paragraph:
[0145] In an embodiment, the HP56 or HP30 polypeptide is separated from other proteins present in the extracts of Helicobacter cells using SDS-PAGE (see Section 5.3. above) and the gel slice containing HP56 or HP30 polypeptide is used as an immunogen and injected into an animal (e.g. rabbit) to produce antisen containing polyclonal HP56 or HP30 antibodies. The same immunogens can be used to immunize mice for the production of hybridoma lines that produce monoclonal anti-HP56 or HP30 antibodies. In particular embodiments, the immunogen is a PAGE slice containing isolated HP56 or HP30 from any Helicobacter strain, including, but not limited to, Helicobacter pylori or Helicobacter felis. Particularly preferred are the strains Helicobacter pylori ATCC:43504, 43504D, 43526, 49503, 51652, 51653, 51932, 700392, 700392D 700824D, 51110, 51111, 51407, 51652, 51653, 700392, 700392D, 43504, 43504D, 43526, 43579, 49503, 51110, 51111, 51407, 51211, 51480, 51482, 51630, 51631, 51632, 51800, 51801, 51802, 51863, 51864, 700030, 700031, 700242, 700932, 49286, 49396, 49615, 51101, 51102, 51103, 51104, 51212, 51401, 51402, 51448, 51449, 51450, 51478, 51480, 51482, 51630, 51632, 51800, 51801, 51802, 51863, 51864, 51932, 700030, 700031, 700242, 700824D and 700932.

Detail Description Paragraph:
[0147] In yet another embodiment, for the production of antibodies that specifically bind one or more epitopes of the native HP56 or HP30 polypeptide, intact Helicobacter or Helicobacter cell lysate are used as immunogen. The cells may be fixed with agents such as formaldehyde or glutaraldehyde before immunization. See Harlow and Lane, 1988, Antibodies:A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., Chapter 15. It is preferred that such anti-whole cell antibodies be monoclonal antibodies. Hybridoma lines producing the desired monoclonal antibodies can be identified by using purified HP56 or HP30 polypeptide, intact Helicobacter cells, Helicobacter cell lysates prepared therefrom or cells expressing Helicobacter antigens as the screening ligand. The immunogen for inducing these antibodies are whole cells, extracts or lysates of any Helicobacter, including, but not limited to, Helicobacter pylori or Helicobacter felis. Preferred species are 43504D, 43526, 49503, 51652, 51653, 51932, 700392, 700392D 700824D, 51110, 51111, 51407, 51652, 51653, 700392, 700392D, 43504, 43504D, 43526, 43579, 49503, 51110, 51111, 51407, 51211, 51480, 51482, 51630, 51631, 51632, 51800, 51801, 51802, 51863, 51864, 700030, 700031, 700242, 700932, 49286, 49396, 49615, 51101, 51102, 51103, 51104, 51212, 51401, 51402, 51448, 51449, 51450, 51478, 51480, 51482, 51630, 51632, 51800, 51801, 51802, 51863, 51864, 51932, 700030, 700031, 700242, 700824D and 700932.

Detail Description Paragraph:
[0148] Polyclonal antibodies produced by Helicobacter cell immunizations contain antibodies that bind other Helicobacter proteins ("non-anti-HP56 or HP30 antibodies") and thus are more cumbersome to use where it is known or suspected that the sample contains other Helicobacter proteins or materials that are cross-reactive with these other proteins. Under such circumstances, any binding by the anti-whole cell antibodies of a given sample or band must be verified by coincidental binding of the same sample or band by antibodies that specifically bind HP56 or HP30 polypeptide (e.g., anti-HP56, anti-HP30, anti-HP56 derived and/or anti-HP30-derived polypeptide), or by competition tests using anti-HP56, anti-HP30, anti-HP56 derived and/or anti-HP30 as the competitor (i.e., addition of anti-HP56 antibodies, anti-HP30 antibodies, HP56 derived polypeptide, HP30-derived polypeptide to the reaction mix lowers or abolishes sample binding by anti-whole cell antibodies). Alternatively, such polyclonal antisera, containing "non-anti-HP56 or HP30" antibodies, may be cleared of such antibodies by standard approaches and methods. For example, the non-anti-HP30 or HP56 antibodies may be removed by precipitation with cells of Helicobacter strains known not to have the HP56 or HP30 polypeptide; or by absorption to columns comprising such cells or cell lysates of such cells.

Detail Description Paragraph:

[0256] In ELISA assays, the protein is immobilized onto a selected surface, for example, a surface capable of binding proteins such as the wells of a polystyrene microtiter plate. After washing to remove incompletely absorbed protein, a nonspecific protein solution that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific absorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

CLAIMS:

1. An isolated HP30 or HP56 polypeptide of Helicobacter spp, wherein the HP30 has a molecular weight of 30 kDa and specifically kinds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 4 or 48 and the HP56 kDa has a molecular weight of 56 kDa and specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 2, said molecular weight as determined in SDS polyacrylamide gel electrophoresis.

2. The HP30 or HP56 polypeptide of claim 1, wherein the Helicobacter spp. is selected from the group consisting of Helicobacter pylori and Helicobacter felis.

3. The HP30 or HP56 polypeptide of claim 2, wherein the Helicobacter spp is Helicobacter pylori.

33. An isolated nucleic acid molecule comprising a nucleotide sequence of Helicobacter spp. encoding an isolated HP30 or HP56 polypeptide or an at least 6 amino acid fragment thereof, of Helicobacter spp, wherein the HP30 has a molecular weight 30 kDa and specifically kinds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 4 or 48 and HP56 has a molecular weight of 56 kDa and specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 2, said molecular weights as determined in SDS polyacrylamide gel electrophoresis or fragment of said nucleic acid which encodes a polypeptide that specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 2 or 4 or 48.

34. An isolated nucleic acid molecule comprising the sequence of SEQ ID NO: 1 or 3 or 47, an at least 18 nucleotide fragment thereof of Helicobacter spp which fragment encodes a polypeptide that specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 2 or 4 or 48, or the complement thereof.

41. A vaccine comprising one or more of an isolated HP30 or HP56 polypeptide of Helicobacter spp. wherein the HP30 has a molecular weight of 30 kDa and specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 4 or 48 and HP56 kDa has a molecular weight of 56 kDa and specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 2, and specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 4 or 48 said molecular weights as determined in SDS of polyacrylamide gel electrophoresis; or an isolated nucleic acid comprising a nucleotide sequence encoding an HP30 or HP56 polypeptide Helicobacter spp. wherein the HP30 has a molecular weight of 30 kDa and specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 4 or 48 and HP56 kDa has a molecular weight of 56 kDa and specifically binds to an antibody that specifically binds to a protein having the sequence of SEQ ID NO: 2, said molecular weights as determined in SDS of polyacrylamide gel electrophoresis, said vaccine further comprising one or more adjuvants or immunostimulatory compounds selected from the group consisting of alum, mLT, QS21, MF59, CpG, DNA, PML, calcium phosphate, calcium sulfate dihydrate, PLG, CT, LTB and CT/LT.

59. An isolated recombinant HP30 or HP56 polypeptide of Helicobacter spp. produced

by a method comprising culturing the transformed host cell of claim 56 under conditions suitable for expression of said HP30 or HP56 polypeptide and recovering said HP30 or HP56 polypeptide.

62. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the polypeptide of claim 1 or 4.

63. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the polypeptide fragment of claim 6.

64. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the isolated fusion polypeptide of claim 8.

65. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 30.

66. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 31.

67. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 32.

68. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 37.

69. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 41.

70. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 15.

71. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering to a subject in need of such prevention, treatment or amelioration, an effective amount of one or more vaccines of claims 15, 20, 25, 30, 31, 36, 41, 42 or 43, each optionally comprising one or more immunogens selected from the group consisting of a lipid, lipoprotein, phospholipid, lipoligosaccharide, protein, attenuated organism and inactivated whole cell, wherein said vaccines are administered simultaneously or sequentially.

72. The method of claim 71 which further comprises administering one or more antibiotics which has Helicobacter bactericidal activity wherein said antibiotic is administered prior to, simultaneously, or sequentially to the administration of said one or more vaccines.

78. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering to a subject in need of such prevention, treatment or amelioration, an effective amount of one or more vaccines of claim 32, each optionally comprising one or more immunogens selected from the group consisting of a lipid, lipoprotein, phospholipid, lipoligosaccharide, protein, attenuated organism and inactivated

whole cell, wherein said vaccines are administered simultaneously or sequentially.

79. The method of claim 78 which further comprises administering one or more antibiotics which has Helicobacter bactericidal activity wherein said antibiotic is administered prior to, simultaneously, or sequentially to the administration of said vaccine.

4. Document ID: US 20040115736 A1

L4: Entry 4 of 17 File: PGPB Jun 17, 2004

DOCUMENT-IDENTIFIER: US 20040115736 A1
TITLE: Immunoassay for H. pylori in fecal specimens using genus specific monoclonal antibody

Abstract Paragraph:
The present invention provides a method for detecting H. pylori in fecal specimens. The present invention employs an immunoassay using genus specific monoclonal antibody to Helicobacter or Campylobacter on one side of the assay and H. pylori specific antibodies on the other side of the assay. The immunoassay will typically be supplied in the form of a kit including a plate of antibody-coated wells, sample diluent, the labeled antibody, e.g., an enzyme-antibody conjugate, wash buffer and, in the case of the ELISA, a substrate solution. Alternatively, one or more of the following assays can be used to detect the presence of the H. pylori antigen: an enzyme-linked assay, a radioimmunoassay, a fluorescence immunoassay, a chemiluminescent assay, a lateral flow assay, an agglutination assay, a particulate-based assay, an immunoprecipitation assay and an immunoblotting assay.

Summary of Invention Paragraph:
[0005] Several major antigens have been identified and used in immunoassays in the detection of H. pylori antibodies. However, these assays have not exhibited the specificity and sensitivity that are desired in serodiagnosis. Newwell, D. G., et al. Serodian. Immunother. Infec. Dis., 3:1-6 (1989). One problem with these immunoassays is cross-reactivity. Studies of the dominant antigens in H. pylori, in particular, the putative flagellar protein, which has a molecular weight of 60 Da, have shown that some of these antigen are not specific to H. pylori and also found in other bacteria such as C. jenui and C. coli. A second problem that has been encountered in designing immunoassays for H. pylori is strain variation. Substantial differences in the antigens have been observed in different strains of H. pylori. These problems preclude designing an assay around the use of a single antigen. One approach that has been taken to improving the specificity and selectivity of antibody immunoassays for H. pylori has been to use a mixture of antigens from different H. pylori strains which mixture is enriched with certain antigen fragments. One ELSIA which detects H. pylori antibodies in blood sera is commercially available from Meridian Diagnostics. This assay uses a bacterial whole cell lysate as the antigen.

Summary of Invention Paragraph:
[0026] H. pylori cells from ATCC strain 43504 have been found to be particularly useful in producing the polyclonal antibody. As previously mentioned, substantial strain variation has been observed in H. pylori. Differences in the organism have

been observed in different geographic regions as well as dietary groups. However, antibodies obtained through sensitization using cells from strain 43504 have been found to be useful in detecting the organism across geographic regions and dietary groups. If necessary, for example, if it is found that the ELISA is not effective in detecting the organism in certain populations, cells from more than one strain of H. pylori could be used to produce the antibody.

Summary of Invention Paragraph:
[0029] To prepare the fecal specimen for use in the assay, the specimen is dispersed in a protein-based sample diluent. The diluent being formulated and buffered to minimize cross-reactivity. As examples of sample diluents, mention can be made of fetal bovine serum, normal goat serum, guinea pig serum, horse serum, casein, albumin, gelatin, and bovine serum albumin (BSA). A dilution of one part fecal specimen and four parts diluent has been found to be useful. In addition to using the protein based additives, cross-reactivity can be reduced by the addition of detergents and increasing or decreasing pH or ionic strength of the diluent buffer. For example, many sample diluents contain Triton X-100 and/or Tween 20 at concentrations ranging between 0.05% and 2%. NaCl can be added in the ranges between 0-2.9% to alter the ionic strength of the buffer system. These changes lead to greater specificity by reducing the likelihood of weak or non-specific interactions from forming.

Summary of Invention Paragraph:
[0030] Cross-reactivity can also be addressed in the formulation of the H. pylori specific antibody solutions and the washes that are used in the assay. The H. pylori specific antibody can be provided in a buffered solution in conjunction with one of the protein sera mentioned previously. The washes used in the assay can be formulated and buffered by the addition of salts and surfactants to control cross-reactivity. A preferred wash for reducing cross-reactivity is a phosphate buffered saline solution.

Detail Description Paragraph:
[0033] H. pylori (ATCC strain 43504) was streaked for isolation on Tryptic Soy Agar (TSA) supplemented with 5% defibrinated sheep blood. The plate was incubated at 37.degree. C. in a microaerophilic environment for 6-7 days. The resultant bacterial growth was evaluated by use of colony morphology, urease, catalase and oxidase reactions, and gram strain. Acceptable growth was subcultured to four TSA with sheep blood agar plates and grown at 37.degree. C. in a microaerophilic environment for 3-4 days.

Detail Description Paragraph:
[0046] So-called triple sandwich assays can also be used for detecting H. pylori in fecal specimens in accordance with the invention. Triple assays are known in the art and the basic methodology can be applied to the detection of H. pylori in fecal specimens. A triple assay is typically conducted by dispersing a fecal specimen suspected of carrying H. pylori in a sample diluent which minimizes cross-reactivity and adding the diluted sample to an immobilized genus specific monoclonal antibody. The sample is incubated to form the antibody-antigen complex. After washing excess specimen from the immobilized support, and H. pylori specific antibody known as a primary antibody and obtained from a species of an antibody producing animal is added to the antibody-antigen complex and incubated for form an antibody-antigen-antibody complex. After forming this complex and removing the unreacted antibody, the complex is reacted with an antibody known as a secondary antibody which is an antibody to the antibody producing species such as anti-(rabbit, cow or goat) immunoglobulin. The secondary antibody is labeled in a conventional manner, typically with an enzyme, an incubated with the antibody-antigen-antibody complex to form a triple antibody complex or sandwich. After removing the unreacted secondary antibody, the antigen is assayed in a conventional manner. In using an enzyme label, a substrate is added to the complex of the antigen and the three antibodies and the reaction of the substrate with the linked

enzyme is monitored to determine the amount of the antigen present in the specimen. In the triple sandwich assay, as in the basic sandwich assay, the washes and the antibody solutions are formulated or buffered to control cross-reactivity as needed.

CLAIMS:

1. A process for the determination of H. pylori antigen in a human fecal specimen which comprises: (a) dispersing human fecal specimen in a sample diluent; 1. contacting the fecal specimen in the diluent with a first antibody to form a complex of the antibody and the antigen; 2. separating said specimen and said complex; 3. exposing the complex to a second antibody and a portion of the second antibody reacting with said complex, one of said first and second antibody being selected from the group consisting of polyclonal H. pylori antigen specific antibodies, a plurality of monoclonal H. pylori antigen specific antibodies and mixtures thereof; and the other of the first and second antibody being a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and also binds to H. pylori antigen, one of said first and second antibody being bound to a solid carrier and the other being labeled with a detection agent; and 4. detecting the amount of the labeled antibody in said complex and in turn determining the presence of H. pylori antigen in said fecal specimen.

10. The process of claim 1 wherein after exposing the complex to the second antibody, the complex is washed with a buffer that reduces cross-reactivity or otherwise improves the specificity of the assay.

11. A process for the determination of H. pylori in a fecal specimen which comprises: (a) dispersing a human fecal specimen in a diluent; (b) contacting the fecal specimen in the diluent with a first antibody reactive with H. pylori antigen bound to a solid carrier and a second labeled antibody reactive with H. pylori to form a complex of the antibodies and the antigen, one of said first and second antibody being selected from the group consisting of polyclonal H. pylori antigen specific antibodies, a plurality of H. pylori antigen specific monoclonal antibodies, and mixtures thereof and the other of the first and second antibody being a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and also binds to H. pylori antigen; (c) separating said specimen and said complex; (d) detecting the labeled antibody in said complex formed in step (b) and in turn determining the presence of H. pylori antigen in said fecal specimen.

12. A process for the determination of H. pylori in a fecal specimen which comprises: (a) dispersing a human fecal specimen in a sample diluent; (b) contacting the fecal specimen in the diluent with a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and binds to H. pylori antigen bound to a solid carrier to form a complex of the antibody and the antigen; (c) separating said specimen and said complex; (d) contacting the antibody-antigen complex formed in step (b) with a primary antibody specific for H. pylori antigen obtained from an antibody-producing species to produce an antibody-antigen-antibody complex; (e) removing the primary antibody not present in the complex from step (c); (f) contacting the antibody-antigen-antibody complex formed in step (e) with a secondary antibody, said secondary antibody being an antibody that specifically binds the antibody-producing species antibody, whereby said secondary antibody forms a complex with said antibody-antigen-antibody complex; and (g) determining the presence of H. pylori antigen in said fecal specimen by detecting the complex formed in step (f).

13. A kit for the determination of H. pylori in a fecal specimen including a plate of wells having bound thereto a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and also binds to H.

pylori antigen, a protein-based sample diluent and a plurality of labeled antibodies selected from the group consisting of polyclonal H. pylori antigen specific antibodies, a plurality of monoclonal H. pylori antigen specific antibodies and mixtures thereof.

15. A process for the determination of H. pylori in a fecal specimen which comprises: (a) dispersing a human fecal specimen in a sample diluent; (h) contacting the fecal specimen in the diluent with a genus directed monoclonal antibody that reacts with different species and strains of Helicobacter or Campylobacter and binds to H. pylori antigen bound to a solid carrier to form a complex of the antibody and the antigen; (i) separating said specimen and said complex; (j) contacting the antibody-antigen complex formed in step (b) with a primary antibody for H. pylori antigen obtained from an antibody-producing species to produce an antibody-antigen-antibody complex; (k) removing the primary antibody not present in the complex from step (c); (l) contacting the antibody-antigen-antibody complex formed in step (e) with a secondary antibody, said secondary antibody being an antibody that specifically binds the antibody-producing species antibody, whereby said secondary antibody forms a complex with said antibody-antigen-antibody complex; and (m) determining the presence of H. pylori antigen in said fecal specimen by detecting the complex formed in step (f).

5. Document ID: US 20030180330 A1

L4: Entry 5 of 17 File: PGPB Sep 25, 2003

DOCUMENT-IDENTIFIER: US 20030180330 A1
TITLE: Method for identifying helicobacter antigens

Abstract Paragraph:
The present invention relates to a method for characterizing or identifying proteins which are expressed by cultivated Helicobacter cells and which preferably react with human antisera. Thus, novel Helicobacter antigens are provided which are suitable as targets for the diagnostis, prevention or treatment of Helicobacter infections.

Summary of Invention Paragraph:
[0019] In a preferred embodiment, the method of the invention further comprises as step (d) the determination of the reactivity of the proteins with antisera. Preferably, these antisera are human antisera which may be derived from Helicobacter positive patients, from patients which are suffering from Helicobacter-mediated diseases such as stomach adenocarcinoma patients, and/or from Helicobacter negative control persons. By screening the reactivity of the proteins with a plurality of antisera, particularly from Helicobacter positive patients, cross-reacting antigens may be identified.

Detail Description Paragraph:
[0071] SDS-PAGE is a common method for the detection of antigens. Unfortunately its resolution power is optimal for protein mixtures of up to only 100 protein species. Therefore a clear assignment to a certain protein species is often not possible if the expected complexity Is above 100. H. pylori extracts contain at least 1800 protein species (FIG. 1), therefore, high-resolution 2-DE is required to detect and

extract by two-dimensional gel electrophoresis, and (c) characterizing and/or Identifying said proteins.

11. The proteins of claim 9 or 10 which are associated with a specific Helicobacter-mediated disease.

17. The use of the proteome or the proteins of any one of claims 3 to 16 for the identification of targets for the diagnosis, prevention or treatment of Helicobacter infections and Helicobacter-mediated diseases.

21. A method for characterizing or identifying proteins which are expressed by Helicobacter cells, comprising the steps: (a) providing a cell extract from Helicobacter cells comprising solubilized proteins, (b) separating said cell extract by two-dimensional gel electrophoresis, and (c) characterizing and/or identifying said proteins.

31. The method of claim 30, wherein said human antisera are derived from Helicobacter positive patients.

32. The method of claim 30 or 31, wherein said human antisera are derived from patients suffering from Helicobacter-mediated diseases.

33. The method of claim 30 or 32, wherein said human antisera are derived from Helicobacter negative control persons.

34. The method of any one of claims 21 to 33, further comprising the steps: (e) repeating steps (a) to (c) and, optionally, (d) with Helicobacter cells from at least one different strain and/or with Helicobacter cells grown under different conditions, and (f) comparing the proteins from different Helicobacter strains and/or from Helicobacter strains grown under different conditions.

35. The method of any one of claims 21 to 34, wherein the Helicobacter cells are cultivated in vitro.

36. The method of any one of claims 21 to 34, wherein the Helicobacter cells are cultivated in vivo.

37. The method of any one of claims 21 to 36, wherein the Helicobacter cells are cultivated at a pH in the range from about 5 to 8.

38. A method for identifying and providing a substance capable of modulating the activity of Helicobacter protein of any one of claims 6-16 comprising contacting said substance with said protein and determining the modulating activity of said substance.

6. Document ID: US 20030166027 A1

L4: Entry 6 of 17 File: PGPB Sep 4, 2003

DOCUMENT-IDENTIFIER: US 20030166027 A1
TITLE: Compositions, test kits and methods for detecting helicobacter pylori

Summary of Invention Paragraph:

[0006] Serological tests and the .sup.13C UBT are the two non-invasive techniques, used in the management of H. pylori infection and eradication. The accuracy of a serological test is dependent on the nature of the antigen(s). Most of the serological tests are ELISA based and use whole cell lysates of H. pylori as the antigen, often in combination with a more purified antigen preparation like recombinant vacA, cagA and/or iceA protein. Using a crude lysate preparation of the whole organism can cause problems with the specificity of the test via nonspecific binding of antibodies not specific for H. pylori to components of the antigen preparation that might be present in other H. pylori related organisms (false positives). On the other hand crude antigen preparation might cause false negative results because unwanted components in the preparation might dilute specific antigens or interfere with the presentation of those required to determine infection. The use of a total protein isolate also prevents serology from detecting loss of the organism and therefore is not suitable for evaluating success of eradication therapy. The UBT gives false negatives when patients are taking proton pump inhibitor drugs (PPI's) due to inhibition of urease activity by neutral pH.

Brief Description of Drawings Paragraph:
[0013] FIG. 1A is titled "Reactivities of H. pylori positive sera with antigens from Hp504" and 1B is titled "Reactivities of H. pylori positive sera with antigens from Hp504." These figures show the average titers of specific antibodies, expressed as percent Integrated Optical density (IOD), with HP1, HP2, HP3 and HP4 from H. pylori strain Hp504 (ATCC#43504) present in sera from 9 patients diagnosed with a H. pylori infection achieved in two independent experiments. The serum samples from each patient were obtained before, 3 months and 5 months after eradication therapy.

Detail Description Paragraph:
[0062] H. pylori strain ATCC#43504 (Hp504)(American Type Culture Collection, Rockville, Md.) and two clinical isolates, Hp08 and Hp02, were used as the source of H. pylori proteins. As a control for the specificity of the serological reactivities Campylobacter jejuni strain ATCC#29428 was included into the experiments.

Detail Description Paragraph:
[0097] The following figures show the serial changes in titers of serum IgG expressed in % of integrated optical density that is left 3 months and 5 months after eradication treatment in comparison to the amount before treatment. The nine sera from with H. pylori infected and treated patients were tested on whole cell lysate separations of H. pylori strain ATCC#43504 (Hp504), Hp08 and Hp02 (clinical isolates). The results are shown for each tested bacterial strain separately to demonstrate that the accuracy of the test is independent of the source of the antigen.

CLAIMS:

1. A composition comprising at least three proteins, wherein the proteins is selected from the group consisting of HP1, HP2, HP3 and HP4, each of said proteins comprising regions which act as antigens specific to Helicobacter pylori, HP1 having of molecular weight of 32 kd, HP2 having of molecular weight of 30 kd, HP3 having of molecular weight of 23 kd, and HP4 having of molecular weight of 15 kd, each of said proteins being derived from Helicobacter pylori bacteria.

12. A method for the preparation of a composition according to claim 10 by preparing a lysate of whole bacterial cell preparations of Helicobacter pylori, subjecting the lysate to gel separation and transferring the proteins to the membranes.

13. A method according to claim 12 wherein the Helicobacter pylori is Helicobacter pylori strain ATCC#43504.

15. A method for detecting the presence of antibodies resulting from Helicobacter pylori infection in a biological sample, the method comprising contacting the sample with a composition according to claim 2, permitting the sample and said composition to form an antigen-antibody complex with respect to any antibody contained in the sample which specific to the antigens included in the proteins of the composition; detecting the presence of any formed antigen-antibody complex denoting the presence of Helicobacter pylori infection.

20. A kit for determining the presence of antibodies formed in response to Helicobacter pylori infection in a biological sample, the kit comprising a composition according to claim 2.

25. In a method for determination the eradication of Helicobacter pylori the improvement consisting in the detection of the presence or absence of antibodies resulting from Helicobacter pylori infection by a method according to claim 15, before, during and after eradication treatment.

26. A method of using a combination of at least 3 proteins from Helicobacter pylori for detecting the presence or absence of antibodies resulting from Helicobacter pylori infection wherein the proteins are selected from the group consisting of HP1, HP2, HP3 and HP4, each of said proteins comprising regions which act as antigens specific to Helicobacter pylori, HP1 having of molecular weight of 32 kd, HP2 having of molecular weight of 30 kd, HP3 having of molecular weight of 23 kd, and HP4 having of molecular weight of 15 kd, each of said proteins being derived from Helicobacter pylori bacteria.

7. Document ID: US 20030148411 A1

L4: Entry 7 of 17 File: PGPB Aug 7, 2003

DOCUMENT-IDENTIFIER: US 20030148411 A1
TITLE: Method for detecting helicobacter pylori and heilmanii in fecal and salivary specimen and biopsy material

Abstract Paragraph:
The invention relates to a method for detecting pathogenic organisms, in particular Helicobacter pylori and H.heilmanii, in fecal, salivary and secretory samples by means of a double-antibody sandwich assay. The inventive method is characterized by dissolving or dispersing the sample having the pathogen antigen in a buffer solution and contacting the buffer solution with a solid phase to which at least two primary antibodies are bound, one of which specifically binds to the pathogenic antigen and the other to human immunoglobulin A; washing the solid phase of non-specifically bound proteins and contacting the solid phase with a secondary antibody which specifically binds to pathogenic antigen, and determining the quantity of specifically bound secondary antibodies.

Detail Description Paragraph:
[0024] Detection of binding: Into the wells there was dosed in each case 100 .mu.l biotin-conjugated polyclonal rabbit-anti-H.pylori antibody (1:10000; DAKO, Hamburg) or horseradish peroxidase (HRP)-conjugated polyclonal goat-anti-H.pylori antibody

(KPL, Kirkegaard and Perry Laboratories, Gaithersburg, MD; a mixture of polyclonal antibodies against the H.pylori types ATCC 43504, 43526, 43579) diluted 1:1000 in washing buffer, and incubated at room temperature for 1 hour while been vibrated. The solution was removed from the wells and each well washed five times with 200 .mu.l washing buffer.

Detail Description Paragraph:
[0031] The result of the mass screening is shown in FIG. 3 in a bar chart. The bar chart shows that patients with an H.pylori infection present in the saliva as a rule have more than 60 ng/ml H.pylori antigens. The testing of the saliva is thus suitable for the detection of the presence of an H.pylori infection. H.pylori antigen concentrations below 25 ng per ml saliva indicate, in contrast, a non-specific cross-reaction with other pathogens.

CLAIMS:

5. Method according to any of claims 1 to 4, wherein the first primary antibody and the secondary antibody bind Helicobacter pylori antigens.

6. Method according to any of claims 1 to 5, wherein the first primary antibody is a mixture of polyclonal and/or monoclonal antibodies against various Helicobacter pylori types.

7. Method according to any of claims 1 to 6, wherein the secondary antibody is a mixture of polyclonal and/or monoclonal antibodies against various Helicobacter pylori types.

9. Method according to any of claims 1 to 4, characterized in that the pathogen is Helicobacter heilmanii.

8. Document ID: US 20020107368 A1

L4: Entry 8 of 17 File: PGPB Aug 8, 2002

DOCUMENT-IDENTIFIER: US 20020107368 A1
TITLE: Helicobacter proteins, gene sequences and uses thereof

Abstract Paragraph:
The invention discloses Helicobacter HP30 or HP56 polypeptide, polypeptides derived thereof (HP30-derived or HP56-derived polypeptides), nucleotide sequences encoding said polypeptides, antibodies that specifically bind the HP30, HP56, HP30-derived or HP56-derived polypeptides and T cells specific for HP30, HP56, HP30-derived or HP56-derived polypeptide. Also disclosed are prophylactic or therapeutic compositions, including immunogenic compositions, e.g. vaccines, comprising HP30, HP56, HP30-derived or HP56-derived polypeptides, nucleic acids encoding the same or antibodies thereto. The invention additionally discloses methods of inducing in animals an immune response to Helicobacter cells.

Summary of Invention Paragraph:
[0018] Preferably, the HP30- or HP56-derived polypeptides of the invention are immunologically cross-reactive with the H. pylori peptide protein from which they

are derived, and are capable of eliciting in an animal an immune response to H. pylori. A preferred HP30- or HP56-derived polypeptide of the invention induces IgM, IgG, IgA, IgE antibodies, a delayed hypersensitivity T cell response and/or cytotoxic T cell response to cells expressing H. pylori antigen (including but not limited to antigen presenting cells such as macrophages, dendritic cells, B cells, or synthetic antigen presenting cells which display H. pylori antigen), native HP30 or HP56 protein from which the polypeptide is derived, H. pylori cells, or H. pylori cell lysate.

Detail Description Paragraph:
[0084] Strains from any of these organism may be obtained worldwide from any biologicals depository, particularly ATCC deposited strains of Helicobacter 43504, 43504D, 43526, 49503, 51652, 51653, 51932, 700392, 700392D 700824D, 51110, 51111, 51407, 51652, 51653, 700392, 700392D, 43504, 43504D, 43526, 43579, 49503, 51110, 51111, 51407, 51211, 51480, 51482, 51630, 51631, 51632, 51800, 51801, 51802, 51863, 51864, 700030, 700031, 700242, 700932, 49286, 49396, 49615, 51101, 51102, 51103, 51104, 51212, 51401, 51402, 51448, 51449, 51450, 51478, 51480, 51482, 51630, 51632, 51800, 51801, 51802, 51863, 51864, 51932, 700030, 700031, 700242, 700824D and 700932.

Detail Description Paragraph:
[0131] The HP56 or HP30 polypeptide of the invention may be isolated from a protein extract including a whole cell extract, of any Helicobacter spp., including, but not limited to, Helicobacter pylori or Helicobacter felis. Strains from any of these organisms may be obtained worldwide from any biologicals depository, particularly strains of ATCC 43504D, 43526, 49503, 51652, 51653, 51932, 700392, 700392D 700824D, 51110, 51111, 51407, 51652, 51653, 700392, 700392D, 43504, 43504D, 43526, 43579, 49503, 51110, 51111, 51407, 51211, 51480, 51482, 51630, 51631, 51632, 51800, 51801, 51802, 51863, 51864, 700030, 700031, 700242, 700932, 49286, 49396, 49615, 51101, 51102, 51103, 51104, 51212, 51401, 51402, 51448, 51449, 51450, 51478, 51480, 51482, 51630, 51632, 51800, 51801, 51802, 51863, 51864, 51932, 700030, 700031, 700242, 700824D and 700932.

Detail Description Paragraph:
[0142] In an embodiment, the HP56 or HP30 polypeptide is separated from other proteins present in the extracts of Helicobacter cells using SDS-PAGE (see Section 5.3. above) and the gel slice containing HP56 or HP30 polypeptide is used as an immunogen and injected into an animal (e.g. rabbit) to produce antisera containing polyclonal HP56 or HP30 antibodies. The same immunogens can be used to immunize mice for the production of hybridoma lines that produce monoclonal anti- HP56 or HP30 antibodies. In particular embodiments, the immunogen is a PAGE slice containing isolated or purified HP56 or HP30 from any Helicobacter strain, including, but not limited to, Helicobacter pylori or Helicobacter felis. Particularly preferred are the strains Helicobacter pylori ATCC:43504, 43504D, 43526, 49503, 51652, 51653, 51932, 700392, 700392D 700824D, 51110, 51111, 51407, 51652, 51653, 700392, 700392D, 43504, 43504D, 43526, 43579, 49503, 51110, 51111, 51407, 51211, 51480, 51482, 51630, 51631, 51632, 51800, 51801, 51802, 51863, 51864, 700030, 700031, 700242, 700932, 49286, 49396, 49615, 51101, 51102, 51103, 51104, 51212, 51401, 51402, 51448, 51449, 51450, 51478, 51480, 51482, 51630, 51632, 51800, 51801, 51802, 51863, 51864, 51932, 700030, 700031, 700242, 700824D and 700932.

Detail Description Paragraph:
[0144] In yet another embodiment, for the production of antibodies that specifically bind one or more epitopes of the native HP56 or HP30 polypeptide, intact Helicobacter or Helicobacter cell lysate are used as immunogen. The cells may be fixed with agents such as formaldehyde or glutaraldehyde before immunization. See Harlow and Lane, 1988, Antibodies:A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., Chapter 15. It is preferred that such anti-whole cell antibodies be monoclonal antibodies. Hybridoma lines producing the desired monoclonal antibodies can be identified by using

purified HP56 or HP30 polypeptide, intact Helicobacter cells, Helicobacter cell lysates prepared therefrom or cells expressing Helicobacter antigens as the screening ligand. The immunogen for inducing these antibodies are whole cells, extracts or lysates of any Helicobacter, including, but not limited to, Helicobacter pylori or Helicobacter felis. Preferred species are 43504D, 43526, 49503, 51652, 51653, 51932, 700392, 700392D 700824D, 51110, 51111, 51407, 51652, 51653, 700392, 700392D, 43504, 43504D, 43526, 43579, 49503, 51110, 51111, 51407, 51211, 51480, 51482, 51630, 51631, 51632, 51800, 51801, 51802, 51863, 51864, 700030, 700031, 700242, 700932, 49286, 49396, 49615, 51101, 51102, 51103, 51104, 51212, 51401, 51402, 51448, 51449, 51450, 51478, 51480, 51482, 51630, 51632, 51800, 51801, 51802, 51863, 51864, 51932, 700030,700031, 700242, 700824D and 700932.

Detail Description Paragraph:
[0145] Polyclonal antibodies produced by Helicobacter cell immunizations contain antibodies that bind other Helicobacter proteins ("non-anti- HP56 or HP30 antibodies") and thus are more cumbersome to use where it is known or suspected that the sample contains other Helicobacter proteins or materials that are cross-reactive with these other proteins. Under such circumstances, any binding by the anti-whole cell antibodies of a given sample or band must be verified by coincidental binding of the same sample or band by antibodies that specifically bind HP56 or HP30 polypeptide (e.g., anti-HP56, anti-HP30, anti-HP56 derived and/or anti-HP30-derived polypeptide), or by competition tests using anti-HP56, anti-HP30, anti-HP56 derived and/or anti-HP30 as the competitor (i.e., addition of anti-HP56 antibodies, anti-HP30 antibodies, HP56 derived polypeptide, HP30-derived polypeptide to the reaction mix lowers or abolishes sample binding by anti-whole cell antibodies). Alternatively, such polyclonal antisera, containing "non-anti-HP56 or HP30" antibodies, may be cleared of such antibodies by standard approaches and methods. For example, the non-anti-HP30 or HP56 antibodies may be removed by precipitation with cells of Helicobacter strains known not to have the HP56 or HP30 polypeptide; or by absorption to columns comprising such cells or cell lysates of such cells.

Detail Description Paragraph:
[0246] In ELISA assays, the protein is immobilized onto a selected surface, for example, a surface capable of binding proteins such as the wells of a polystyrene microtiter plate. After washing to remove incompletely absorbed protein, a nonspecific protein solution that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific absorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

CLAIMS:

1. An isolated HP30 or HP56 polypeptide of Helicobacter spp, wherein the HP30 has a molecular weight of 30 kDa and the HP56 kDa has a molecular weight of 56 kDa as determined in SDS polyacrylamide gel electrophoresis.

2. The HP30 or HP56 polypeptide of claim 1, wherein the Helicobacter spp. is selected from the group consisting of Helicobacter pylori and Helicobacter felis.

3. The HP30 or HP56 polypeptide of claim 2, wherein the Helicobacter spp is Helicobacter pylori.

33. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an isolated HP30 or HP56 polypeptide or an at least 6 amino acid fragment thereof, of Helicobacter spp, wherein the HP30 has a molecular weight 30 kDa and HP56 has a molecular weight of 56 kDa as determined in SDS polyacrylamide gel electrophoresis or fragment thereof.

41. A vaccine comprising one or more of an isolated HP30 or HP56 polypeptide of

Helicobacter spp. wherein the HP30 has a molecular weight of 30 kDa and HP56 kDa has a molecular weight of 56 kDa as determined in SDS of polyacrylamide gel electrophoresis; or an isolated nucleic acid comprising a nucleotide sequence encoding an HP30 or HP56 polypeptide Helicobacter spp. wherein the HP30 has a molecular weight of 30 kDa and HP56 kDa has a molecular weight of 56 kDa as determined in SDS of polyacrylamide gel electrophoresis said vaccine further comprising one or more adjuvants or immunostimulatory compounds selected from the group consisting of alum, mLT, QS21, MF59, CpG, DNA, PML, calcium phosphate and PLG.

57. An isolated recombinant HP30 or HP56 polypeptide of Helicobacter spp. produced by a method comprising culturing the transformed host cell of claim 54 under conditions suitable for expression of said HP30 or HP56 polypeptide and recovering said HP30 or HP56 polypeptide.

60. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the polypeptide of claim 1.

61. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the polypeptide fragment of claim 6.

62. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the isolated fusion polypeptide of claim 8.

63. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 30.

64. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 31.

65. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 32.

66. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 37.

67. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 41.

68. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering an effective amount of the vaccine of claim 15.

69. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering to a subject in need of such prevention, treatment or amelioration, an effective amount of one or more vaccines of claims 15, 20, 25, 30, 31, 36 or 41, each optionally comprising one or more immunogens selected from the group consisting of a lipid, lipoprotein, phospholipid, lipoligosaccharide, protein, attenuated organism and inactivated whole cell, wherein said vaccines are administered simultaneously or sequentially.

70. The method of claim 69 which further comprises administering one or more antibiotics which has Helicobacter bactericidal activity wherein said antibiotic is administered prior to, simultaneously, or sequentially to the administration of said one or more vaccines.

76. A method of preventing, treating or ameliorating a disorder or disease associated with infection of an animal with Helicobacter by administering to a subject in need of such prevention, treatment or amelioration, an effective amount of one or more vaccines of claim 32, each optionally comprising one or more immunogens selected from the group consisting of a lipid, lipoprotein, phospholipid, lipoligosaccharide, protein, attenuated organism and inactivated whole cell, wherein said vaccines are administered simultaneously or sequentially.

77. The method of claim 76 which further comprises administering one or more antibiotics which has Helicobacter bactericidal activity wherein said antibiotic is administered prior to, simultaneously, or sequentially to the administration of said vaccine.

☐ 9. Document ID: US 20020090660 A1

L4: Entry 9 of 17 File: PGPB Jul 11, 2002

DOCUMENT-IDENTIFIER: US 20020090660 A1
TITLE: Helicobacter pylori antigens in blood

Abstract Paragraph:
The present invention relates to the finding and detection of Helicobacter pylori (H. pylori) antigens in blood of infected individuals. The H. pylori antigens are components of H. pylori cells which include, but not limited to DNA, RNA, and fragments of nucleotides, proteins or peptides. H. pylori DNA, RNA, and fragments of nucleotides can be detected by polymerase chain reaction (PCR), ligase chain reaction (LCR), or DNA hybridization methods or other amplification methods. H. pylori proteins or peptides or other antigenic components thereof can be detected by immunoassays or immunoblot using an antibody against H. pylori, preferably an antibody purified by an affinity column. The present invention further provides immunoassay methods, diagnostic kits, and an immunochromatographic assay device for detection of Helicobacter pylori antigens in serum samples.

Summary of Invention Paragraph:
[0010] U.S. Pat. Nos. 5,716,791, '5,871,942, and 5,932,430 disclose immunoassays for detecting H. pylori antigens in stool specimens using a polyclonal antibody which is obtained from sensitizing animal with H. pylori cells (i.e., ATCC strain 43504). The antibody is purified by DEAE (diethylaminoethyl cellulose) column. Although the stool antigen test is reported to be satisfactory, the collection and process of the stool specimens are found to be difficult and unpleasant. Many patients are unwilling to provide stool samples to physician due to offensive odor and lack convenient collection device.

Summary of Invention Paragraph:
[0019] The primers are prepared based upon conserved sequence found in consensus fragments of H. pylori strains, such as ATCC strains 43504, 43571, 43629, and

49053. The preferred primers range is from 15 to 25 base pairs (bps), most favorably about 20 bps in length. Better amplification can be obtained when both primers (forward and reverse primers) are the same length and with roughly the same nucleotide composition. The preferred blood sample for PCR is plasma.

Summary of Invention Paragraph:
[0020] The LCR method provided in the present invention requires the use of a DNA ligase and two sets of oligonucleotides which are specific to H. pylori. The preferred DNA ligase is Pfu DNA ligase, which is a thermostable DNA ligase isolated from Pyrococcus furiosus and is commercially available. The two sets of oligonucleotides for LCR is preferably longer in length than the primers for PCR. Like the PCR primers, the LCR oligonucleotides are derived from conserved sequence of the consensus fragments of H. pylori strains, such as ATCC strains 43504, 43571, 43629, and 49053.

Summary of Invention Paragraph:
[0034] Furthermore, the dissociation treated serum sample can be treated with a protein based reagent to minimize cross-reactivity. The preferred protein based reagent contains at least one of the following proteins: fetal bovine serum, pig serum, normal goat serum, horse serum, casein, albumin, gelatin, and bovine serum albumin.

Summary of Invention Paragraph:
[0041] H. pylori cells from ATCC strain 43504 have been found to be particularly useful for producing primary antibody against H. pylori in stool samples (See U.S. Pat. No. 5,716,791). That is because the antibodies produced through sensitization using cells from strain 43504 can detect the organism across geographic regions and dietary groups. Other H. pylori strains, such as ATCC 43571, 43629, 49053, have demonstrated similar antigenic capability. Therefore, it is worthwhile to find consensus fragments among these strains. This can be performed by digesting the extracted nucleic acids from the above mentioned H. pylori strains with the same restriction endonuclease(s), followed by running the digested H. pylori nucleic acid fragments through an agarose gel electrophoresis. The consensus fragments can be cut out and extracted. The nucleotide sequences of the consensus fragments can be analyzed. The conserved sequence of the consensus fragments can then be used for designing the primers or oligonucleotides for PCR or LCR.

Detail Description Paragraph:
[0056] H. pylori seed stocks of ATCC strains 43504, 43571, 43629, and 49053 were individually thawed at room temperature and diluted in 5 ml Brucella broth. Immediately after dilution, 0.2 ml of the diluted bacteria suspension were spread on a Trypticase soy blood agar plate, supplemented with 5% sheep blood. Plates were incubated under microaerobic condition for the bacteria to grow. After incubation, colonies were then scraped off the plate and washed two times with PBS. The washed pellets were then suspended in PBS.

Detail Description Paragraph:
[0092] Furthermore, the dissociation treated serum sample can be treated with a protein based reagent to minimize cross-reactivity. The preferred protein based reagent contains at least one of the following proteins: fetal bovine serum, pig serum, normal goat serum, horse serum, casein, albumin, gelatin, and bovine serum albumin.

CLAIMS:

1. A method for detecting Helicobacter pylori antigen in a serum sample comprising: providing said serum sample; treating said serum sample with a dissociation reagent; removing said dissociation reagent; providing a first antibody against Helicobacter pylori, wherein said first antibody is a polyclonal antibody purified by an affinity column; contacting said dissociation reagent-treated serum sample

with the first antibody to form a first complex; providing a second antibody against Helicobacter pylori, wherein said second antibody is a polyclonal antibody purified by an affinity column; wherein one of said first and second antibody is bound to a solid carrier, the other is labeled with a detection agent; contacting said first complex with the second antibody to form a second complex; and detecting the presence of Helicobacter pylori in said second complex by detecting the presence of the detection agent.

26. A method for detecting Helicobacter pylori antigen in a serum sample comprising: providing said serum sample; treating said serum sample at a dissociation condition; wherein said dissociation condition is obtained by changing pH of said serum sample to alkaline or acidic pH or by elevating temperature of said serum sample; and wherein upon completion of said dissociation condition, said serum pH or said elevated serum temperature is returned to original condition; providing a first antibody against Helicobacter pylori, wherein said first antibody is a polyclonal antibody purified by an affinity column; contacting said dissociation condition-treated serum sample with the first antibody to form a first complex; providing a second antibody against Helicobacter pylori, wherein said second antibody is a polyclonal antibody purified by an affinity column; wherein one of said first and second antibody is bound to a solid carrier, the other is labeled with a detection agent contacting said first complex with the second antibody to form a second complex; and detecting the presence of Helicobacter pylori in said second complex by reading the presence of the detection agent.

☐ 10. Document ID: US 20020016289 A1

L4: Entry 10 of 17 File: PGPB Feb 7, 2002

DOCUMENT-IDENTIFIER: US 20020016289 A1
TITLE: METHODS FOR TREATMENT AND PREVENTION OF HELICOBACTER PYLORI INFECTION USING LACTOFERRIN

Summary of Invention Paragraph:
[0018] Despite the great weight of evidence suggesting the antibacterial effect of lactoferrin, the glycoprotein has been shown, prior to the present invention, to assist rather than inhibit the growth of H. pylori. For example, in 1993 Husson et al., investigated the in vitro activity of native human lactoferrin and other iron containing proteins on the growth of 15 isolates and an ATCC reference strain (#43504) of H. pylori in a study of lactoferrin as an iron chelator/iron donator. Husson et al., Infect. Immun., 1993, 61:2694-2697. Evidence suggested that H. pylori acquired iron directly from lactoferrin using a mechanism in which the host lactoferrin was bound directly to bacterial outer membrane receptors and the iron was removed from the protein for subsequent use by the microbe. Husson et al., supra. See also, Herrington and Sparling, Infect. Immun., (1985) 48:248-251 and Neilands, Annu. Rev. Microbiol., (1982) 36:285-309 regarding Haemophilus influenzae and Neisseria spp. Specifically, Husson et al., reported that H. pylori acquired iron from 10 .mu.M (0.78 mg/ml) 30%-iron-saturated human lactoferrin by direct lactoferrin-organism contact and that this iron was available for use by the organism for growth. As controls, the iron from iron-siderophores enterochelin and pyochelin was not available to H. pylori. Iron was also not available from human transferrin, bovine lactoferrin or hen ovolactoferrin. This evidence strongly

indicated that human lactoferrin played a major role in the virulence, rather than control, of H. pylori infections. Additionally, prior to the present invention, lactoferrin has not been shown efficacious against other enteropathogens such as Shigella and Escherichia coli.

Detail Description Paragraph:
[0057] The urea breath test measures the magnitude of global urease activity in the gastrointestinal tract. Graham et al., Am. J. Gastroent., 1991, 86:1118-1122. Orally administered urea, labeled with either the stable isotope .sup.13C, or the radioactive isotope .sup.14C, is hydrolyzed by urease into ammonia and labeled carbon dioxide gas. The labeled gas appears in the expired breath following consumption, and when quantified, provides a straightforward, non-invasive method by which to measure the enzymatic urease activity of the gut. Gastrointestinal urease has been shown to be of microbial and not mammalian origin. And, because H. pylori is the most common urease positive gastric pathogen, an assay specificity and sensitivity of approximately 80% has been obtained for using the urea breath test. The test has provided a convenient measure of the gastrointestinal burden of urease positive microbes and for the evaluation of the effectiveness of prospective therapies used against them. Urea, prepared with the stable isotope .sup.13C is the preferred substrate: it is available at low cost, may be administered repeatedly throughout life without contributing to the radioactive body burden, and is more ethically appropriate for children and pregnant women.

CLAIMS:

8. The method of claim 1 wherein the enteropathogen is selected from the group consisting of Shigella species, Salmonella species, Helicobacter species and E. coli.

11. Document ID: US 6902903 B1

L4: Entry 11 of 17 File: USPT Jun 7, 2005

DOCUMENT-IDENTIFIER: US 6902903 B1
TITLE: Helicobacter pylori diagnostics

Abstract Text (1):
Novel methods, membrane supports and immunodiagnostic test kits for diagnosing Helicobacter pylori infection, are disclosed. The methods can also be used to monitor the progress of treatment of an infection. The methods, supports and kits employ both type-common and type-specific H. pylori antigens and can conveniently be performed in a single-step assay format. The methods provide for highly accurate results and discriminate between H. pylori Type I and H. pylori Type II infection so that an accurate diagnosis can be accomplished.

Brief Summary Text (10):
U.S. Pat. No. 4,882,271 describes an H. pylori assay that utilizes high molecular weight cell-associated proteins, on the order of 300 kDa to 700 kDa, having urease activity, in an enzyme-linked immunosorbent assay (ELISA), in an attempt to circumvent the problems with cross-reactivity.

Brief Summary Text (16):
The present invention provides a simple, extremely accurate and efficient method for diagnosing H. pylori infection, as well as for distinguishing between H. pylori Type I and H. pylori Type II infections. Thus, the method provides a technique for screening for individuals with H. pylori Type I infection. If Type I infection is detected, the individual can be given antibiotics to treat or prevent type B gastritis, peptic ulcers, and gastric tumors. The method is also useful for monitoring the course of treatment in a patient with an H. pylori infection. The assay method utilizes both type-common antigens, as well as particular type-specific antigens from the bacterium.

Brief Summary Text (17):
Accordingly, in one embodiment, the subject invention is directed to a method of detecting H. pylori infection comprising: (a) providing a biological sample; (b) reacting the biological sample with one or more H. pylori type-common antigens and reacting the biological sample with one or more purified type-specific H. pylori Type I antigens, under conditions which allow H. pylori antibodies, when present in the biological sample, to bind with the H. pylori type-common antigens and/or the type-specific antigens, thereby detecting the presence or absence of H. pylori infection.

Brief Summary Text (18):
In other embodiments, the invention is directed to a method for distinguishing between H. pylori Type I and H. pylori Type II infection in a biological sample, or a method of monitoring a subject undergoing therapy for an Helicobacter pylori infection, the methods comprising: (a) immobilizing one or more H. pylori type-common antigens, e.g., an H. pylori lysate and/or H. pylori urease, and immobilizing one or more purified type-specific H. pylori Type I antigens, e.g., H. pylori VacA and/or H. pylori CagA, on a nitrocellulose strip; (b) contacting the nitrocellulose strip from step (a) with the biological sample under conditions which allow anti-H. pylori Type I and anti-H. pylori Type II antibodies, when present in the biological sample, to bind with H. pylori type-common antigens present in the lysate and/or the type-specific H. pylori Type I antigens; (c) removing unbound antibodies; (d) providing a detectably labeled anti-human immunoglobulin antibody; and (e) detecting the presence or absence of bound anti-human immunoglobulin antibodies in the biological sample, thereby detecting the presence or absence of H. pylori Type I or Type II infection.

Brief Summary Text (20):
In yet further embodiments, the invention is directed to membrane supports comprising one or more H. pylori type-common antigens and one or more purified type-specific H. pylori Type I antigens, discretely immobilized thereon.

Brief Summary Text (23):
In other embodiments, the invention is directed to immunodiagnostic test kits for detecting H. pylori infection. The kits comprise (a) one or more H. pylori type-common antigens; (b) one or more purified type-specific H. pylori Type I antigens; and (c) instructions for conducting the immunodiagnostic test.

Brief Summary Text (24):
In still further embodiments, the invention is directed to an immunodiagnostic test kit for distinguishing between H. pylori Type I and H. pylori Type II infection in a biological sample, or for monitoring a subject undergoing therapy for an Helicobacter pylori infection. The test kit comprises (a) one or more H. pylori type-common antigens immobilized on a nitrocellulose strip, e.g., an H. pylori lysate and/or H. pylori urease; (b) one or more purified type-specific H. pylori Type I antigens, e.g., H. pylori VacA and/or H. pylori CagA, immobilized on a nitrocellulose strip; and (c) instructions for conducting the immunodiagnostic test.

Drawing Description Text (2):
FIG. 1 depicts a representative test strip for use in a strip immunoblot assay (SIA). Human IgG is used as an internal control at two different levels (Level I,